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The Effects of Contaminated Sediments on Representative Estuarine Species and Developing Benthic Communities. Chapter 21

University of West Florida, Pensacola

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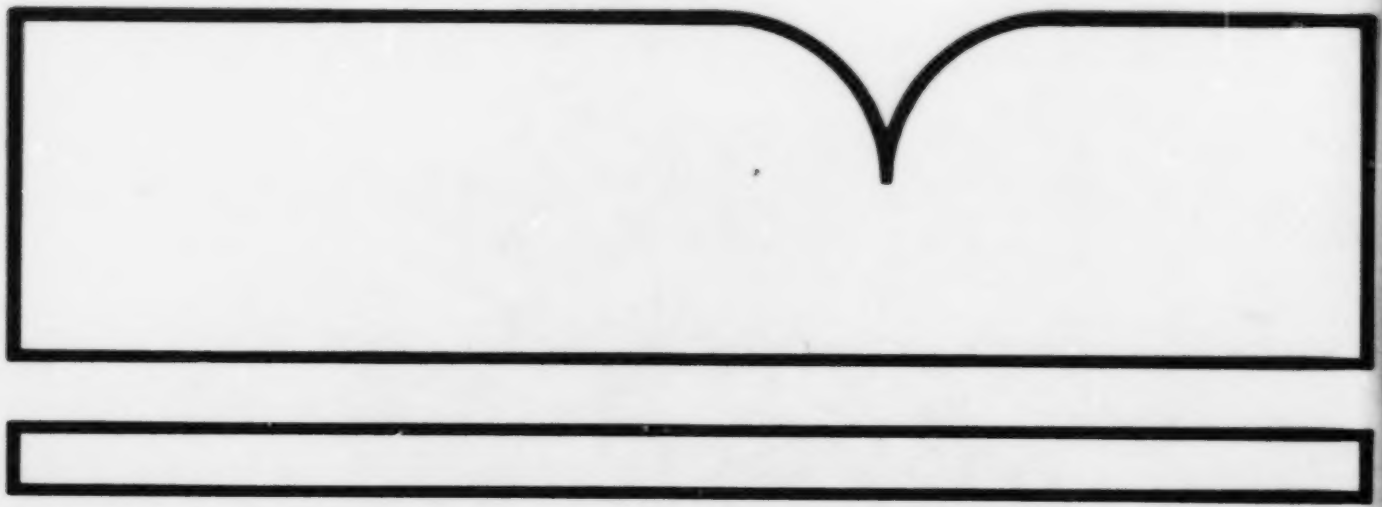

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16. ABSTRACT

Bioassay techniques developed to examine acute and sublethal effects of dredged sediments on marine life are described. Results are reported for laboratory tests conducted to determine sublethal and acute effects of Kepone-sorbed sediment and dredged spoil material from the James River and the Houston Ship Channel on the mysid shrimp, Mysidopsis bahia; oyster, Crassostrea virginica; and polychaete, Arenicola cristata. Criteria established for the study include (1) survival of mysids; (2) shell deposition and bioaccumulation of contaminants; (3) substrate-reworking and bioaccumulation by lugworms; (4) resiliency of macrofaunal organisms that settled onto test sediments from planktonic larvae.

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CHAPTER 21

THE EFFECTS OF CONTAMINATED SEDIMENTS ON REPRESENTATIVE ESTUARINE SPECIES AND DEVELOPING BENTHIC COMMUNITIES

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INTRODUCTION

Many xenobiotics found in the marine environment, e.g., organic pesticides and heavy metals, have a great affinity for particulate material (especially organics) and, in time, become associated with sediments [1,2]. Bottom sediments of coastal waters are frequently resuspended due to a variety of physical and biological phenomena, e.g., local hydrography, wind and wave action, bioturbations by infaunal organisms, and by man's activity, primarily in the form of dredging operations. Dredging and redeposition of spoil material are necessary for maintaining the waterways and harbors of this country. In 1978, more than 190 million cubic meters of dredged material were deposited along the coasts of the United States [3]. Resuspension of sediment could pose a potential hazard to the marine ecosystem if associated pollutants become biologically available to marine organisms.

To ensure that dredging activities are conducted with a minimum degree of risk, all proposed dredging must be evaluated on the basis of criteria established by the U.S. Environmental Protection Agency (EPA), as stated in the *Federal Register*, Vol. 42, No. 7, January 11, 1977. As an aid in implementing these criteria, a joint committee of the U.S. Army Corps of Engi-

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neers (USCE) and the EPA published guidelines [4] for determining the relative impact of dredged material on the marine environment. These guidelines represent the current state-of-the-art for toxic sediment evaluation and are subject to update periodically to reflect improved evaluation procedures. The objective of our research was to develop a flow-through toxicity test (bioassay) to determine biological effects of potentially toxic sediments on representative estuarine organisms and developing benthic communities.

A variety of bioassays have been developed [5,6] to generate data in support of legislative mandates, such as The Marine Protection Research and Sanctuaries Act of 1972 (Public Law 92-532, Section 103A). Bioassays measure the response of an organism exposed to toxicant concentrations over a period of time [6]. When the biological response is death, an LC50 level (the toxicant concentration that is lethal to 50% of the test organisms, usually within 96 hr) is established. If the response is a sublethal or behavioral anomaly, an EC50 level (the toxicant concentration that affects 50% of the test organisms) is determined. Our test incorporates several established bioassay techniques that were modified to examine acute and sublethal effects of dredged sediments on marine life. The test is intended to serve as a screening tool to detect potential hazards of dredge spoils prior to disposal in the marine environment.

Our approach was to simulate and then compare certain aspects of the marine environment before and after deposition of spoil material. We developed small-scale estuarine microcosms in 10-gallon aquaria receiving flowing unfiltered seawater. A number of these aquaria received different concentrations of test sediments (dredge spoils), while others remained unperturbed and served as controls. Comparisons between control and experimental aquaria identified acute and sublethal effects caused by exposure to test sediments.

Organisms selected for this study represent the three environmental compartments affected by dredging activities. Included were: the mysid shrimp, *Mysidopsis bahia*, a water column crustacean that often scavenges on or near bottom sediments; the oyster, *Crassostrea virginica*, an epibenthic mollusk that filters surrounding water for its nourishment; and a deposit-feeding polychaete, *Arenicola cristata* (commonly called the lugworm), that lives directly within bottom sediments and feeds primarily on the overlying detrital layer. Test criteria were developed to identify effects of toxic sediments on: (1) survival of mysids; (2) shell deposition and bioaccumulation of known contaminants by oysters; (3) substrate-reworking and bioaccumulation by lugworms; and (4) the resiliency of the benthic community in terms of numbers and variety of macrofaunal organisms that settled onto test sediments from planktonic larvae within 28 days.

ECOLOGICAL SIGNIFICANCE OF TEST CRITERIA

Mysidopsis bahia is a sensitive indicator of pollutant stress [7]. Representative acute bioassays resulted in the following 96-hr LC50 levels:

cadmium, 15.5 μg/1; Dimilin, 2.06 μg/1; Kepone®, 10.6 μg/1; and Sevin, 7.7 μg/1 [8]. Several commercially significant species, e.g., speckled trout, flounder and striped bass [9-11], utilize mysids as an important diet staple. Thus, mass mortality of mysids could have a serious impact on higher trophic levels.

The oyster, *Crassostrea virginica*, has been used extensively as a bioassay organism because of its commercial importance (1977 landings were valued at $52.5 million) and its sensitivity to a wide variety of toxicants. Further, oysters are sedentary organisms unable to avoid long-term exposure to environmental contaminants. A bioassay developed to measure the effect of pollutants on shell growth [12] demonstrates the relative toxicity of pollutants by comparing the amount of shell material deposited by oysters exposed to a range of toxicant concentrations. Generally, the relationship of shell deposition to toxicant concentration is logarithmic [13]. The EC50 of several toxicants on shell deposition are: dieldrin, 15.0 μg/1; chlordane, 4.7 μg/1; and endrin, 33.0 μg/1 [14]. Mercury also reduces shell growth at concentrations as low as 10 μg/1 [15].

Lugworms are sedentate polychaetes distributed worldwide in most littoral habitats. The European species, *Arenicola marina*, is reported to comprise 20–30% of the benthic biomass from the Balgazand area of the Dutch Wadden Sea [16]. Lugworms rework sediments as a function of their feeding. Populations of *A. marina* have been observed to turn over more than 500 ton/ac/yr of sediment [17]. They inhabit u-shaped burrows at depths of 10–30 cm from which they draw down nutrient-rich surface deposits by ingesting particles at the base of a funnel-shaped feeding column. They also pump overlying water through the burrow for respiration and ventilation. Consequently, the lugworm, like certain other benthic invertebrates [18-20], provides pathways for cycling nutrients, organic material, oxygen and pollutants between the sediment and the water column. Lugworms also provide food for a variety of commercially important bottom-feeding fish [21,22], thereby serving as a significant link in estuarine food webs.

The effect of several pesticides on lugworm substrate reworking rates has been determined [8]. Kepone is toxic to lugworms at 29.5 μg/1 seawater and significantly reduces substrate reworking activity at concentrations as low as 2.8 μg/1 of seawater. Methyl parathion inhibits lugworm activity at concentrations between 75 and 150 μg/1 and sodium pentachlorophenol inhibits lugworm activity at 45–80 μg/1 [23].

Burial and alteration of the physical characteristics of the sediment at the dump site may inhibit benthic community productivity. Consequently, recovery of the benthos will be influenced to a great extent by substratum acceptance by settling planktonic larvae. Typically, larvae of benthic invertebrates have subtle physical, chemical and biological requirements for substratum selection [24,25]. By comparing the numbers and varieties of organisms that settled on test sediments with those that develop on control sediments (found at or near the dump site prior to dredge spoil disposal), it will be possible to determine potential long-term effects on recolonization of

the benthic habitat. This type of test has been used previously to examine the effect of pesticides and drilling muds on benthic community development. Hansen [26] found Aroclor 1254® (a polychlorinated biphenyl) altered the composition of benthic communities at concentrations as low as 0.1 μg/l of seawater. Tagatz [8] found that a variety of drilling mud constituents altered the numbers and diversity of organisms that develop from planktonic larvae.

METHODS

This study was conducted at the EPA Environmental Research Laboratory, Gulf Breeze, Florida, from April through December of 1978.

Our initial experiments concerned methods development. We selected a local pristine area (hereafter referred to as Range Point) as a hypothetical dredge spoil disposal site. Silica quartz sand from Range Point was used as the substrate base in the test aquaria. Sediment collected from an area adjacent to Range Point and spiked with three concentrations of the organochlorine pesticide Kepone was used as the test sediment. The objective of this preliminary experiment was to ensure that the proposed bioassay technique was sufficiently sensitive to elicit a graded response to a range of toxic sediments. Kepone was selected as the toxicant because it is a persistent compound that readily associates with particulate material [27]. Also, Kepone-contaminated sediments currently pose a serious hazard to aquatic life in the James River, Virginia, and Chesapeake Bay areas [28]. Studies with Kepone-sorbed sediment were followed by tests with actual dredge spoil material from the James River and the Houston Ship Channel.

Exposure System

Twelve 10-gallon glass aquaria (50 × 25 × 40 cm) were used as test vessels (three replicates per concentration plus three control replicates) (Figure 1). Aquaria were set up with 10 cm of silica quartz sand (particle size 200–800 μm) and flowing unfiltered seawater. Sand collected along the shore of Santa Rosa Sound was air-dried and sieved to remove debris. A 1-mm layer of finely ground seagrass (predominantly *Thalassia testudinum*) was added to the aquaria to serve as the detrital component of the microcosms. Seawater of ambient temperature and salinity was pumped directly from Santa Rosa Sound to a head box in the laboratory. Water flowed from the head box to two identical splitter boxes (Figure 1). Each splitter box directed seawater to six aquaria. Water entered individual aquaria at a rate of 300 ml/min. Flow to the aquaria was adjusted by raising or lowering individual standpipes in the splitter box. Effluent water drained from the aquaria through openings 25 cm above the substrate on the side of the aquaria opposite the incoming water and was routed to holding ponds. Preliminary tests conducted with dye markers demonstrated complete mixing of incoming water with no stratification.

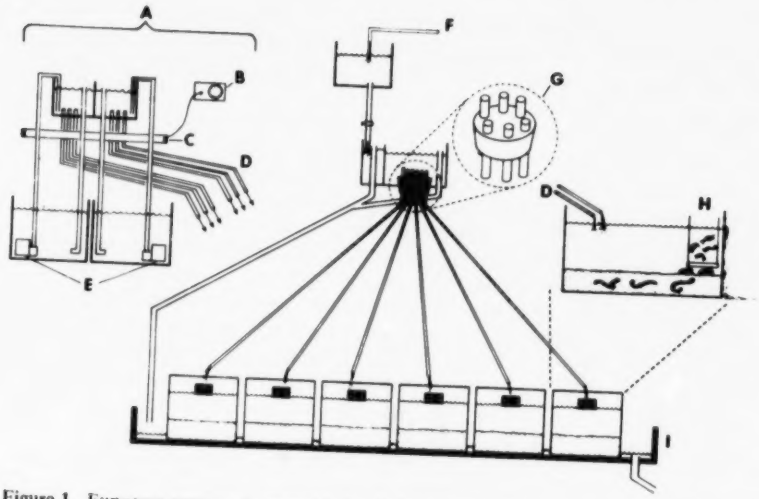


Figure 1. Exposure system: A, suspended sediment dosing apparatus; B, timer; C, crimping bar and solenoids; D, delivery tubes; E, submersible pumps; F, seawater headbox; G, splitter box standpipes; H, mysid cages; I, effluent drain.

Preparation and Delivery of Test Sediment

Test sediment (90% silica sand, 10% silt–clay) was collected from a pristine salt marsh and wet-sieved (0.5 mm mesh) to remove large particles and macrofauna. Kepone (88% pure) dissolved in acetone was mixed with filtered seawater (20 °/oo salinity, 25°C) at nominal concentrations of 0.1, 1.0 and 10.0 mg/l. Test sediment was added to the spiked seawater and mixed with a magnetic stirrer for 48 hours. The mixture was allowed to settle for several hours, the supernate was decanted and the Kepone-sorbed sediment was collected for testing. Kepone rapidly achieves equilibrium with particulate material [29] and theoretically would associate with sediment well within 48 hours. Control sediment was prepared similarly but with acetone carrier only.

Following introduction and acclimation of bioassay organisms in aquaria, flow of seawater was stopped and 600 ml of test sediment of the appropriate concentration was poured uniformly over the surface of the designated aquaria. One hour was allowed for the sediment to settle before the seawater flow was resumed, leaving a 0.5-mm overlay of test material on the silica sand base.

Dredge spoil, like natural sediment, is subject to periodic resuspension. To simulate resuspension in aquaria, we constructed a suspended-sediment dosing apparatus (Figure 1). A 2% sediment mixture (1 liter of sediment per

50 liters of seawater) that contained the desired Kepone concentrations was added to 100-liter reservoir tanks. Sediment was maintained in suspension by continuous mixing with a submersible pump (Little Giant model 2E-38N); a second submersible pump carried the sediment–water mixture to a constant-head box. A standpipe in the constant-head box regulated head pressure and returned overflow water to the reservoir tank. Suspended sediment was metered to the individual test aquaria from the constant-head box through glass tubes (3-mm bore) fitted with a length of flexible silicon hose. The hose, threaded between two aluminum bars, was normally crimped closed by spring tension. Crimping bars were attached to two solenoids. When the solenoids were energized (120 VAC), the crimping bar retracted, allowing the sediment–water mixture to flow to the appropriate aquaria. A timer (Paragon #8025) controlled the frequency and duration of electrical pulse to the solenoids. Adjusting the timer to the desired setting made it possible to accurately control the amount of suspended Kepone-contaminated sediment entering the test aquaria. Control aquaria received a 2% mixture of the control sediment in the same manner.

Resuspension of bottom sediments is commonly a result of tidal scour. For the methods development study we simulated a diurnal tidal cycle by dosing test aquaria with suspended sediment four times daily (at 6-hour intervals). Each pulse delivered 500 ml of the suspended sediment mixture to the aquaria. Sediments remained in suspension in the aquaria for approximately 30 minutes. Suspended solids entering the aquaria were measured weekly.

Bioassays

Kepone-Sorbed Sediment

Each aquarium contained 10 mysids, 5 oysters and 5 lugworms. Mysids and lugworms were obtained from stocks cultured in the laboratory [6,30]. Oysters were collected locally. All animals were maintained for at least one week prior to testing in flowing seawater of the same salinity and temperature as the test water.

Mysids (7–10 days old) were placed in Nitex® screen cages (1000 μm) mesh) suspended in the aquaria. The cages were constructed with petri dish bottoms (9 cm in diameter) and received 15 ml of the appropriate test sediment. Mysids were counted daily for 10 days, and the number of living animals recorded. Mysids were fed *Artemia* larvae daily throughout the test period. The significance of sediment–Kepone concentration to mysid survival was determined by analysis of variance.

Oysters were placed on the substrate of the aquaria. Shell deposition tests were conducted in accordance with procedures outlined by EPA Standard Methods [5]. Oysters (2.5–4.0 cm in height) were brushed clean to remove all epiphytes and the outer shell rim was ground smooth with a disc grinder. The oyster typically responds to removal of the peripheral shell by depositing new shell. The new shell growth is essentially linear for one week; the oyster

then thickens the longitudinal growth. By measuring new growth weekly, then regrinding the outer rim, we could use the rate of shell deposition as an index of the oysters' reactions to their respective sediment Kepone concentration during the 28-day test period. Significance of sediment–Kepone concentration on shell growth was determined by analysis of variance and Neuman Keuls post hoc analysis.

Lugworms (4.0–6.0 cm long) were introduced into each aquarium. We ensured that only "healthy" animals were selected by allowing lugworms 1 minute to burrow into the substrate. Animals that remained on the surface after one minute were replaced. Lugworm activity was monitored by comparing the number of feeding funnels that appeared on the substrate surface.

The test was terminated after 28 days. Lugworms and oysters were collected and analyzed for whole-body Kepone residues according to the method of Schimmel et al. [31]. Animals tested for Kepone residues were placed in uncontaminated seawater for several hours prior to preparation for analysis to remove Kepone superficially sorbed to their integuments.

Macrofauna that developed in sediment from planktonic larvae were obtained by sieving sediment from all aquaria. Organisms retained by a 1-mm mesh were collected, preserved in 60% isopropanol, and identified to species. Numbers of animals in the three replicate aquaria at each dose level were pooled. Comparisons of the numbers and varieties of animals in control and exposed aquaria were determined by analysis of variance and Duncan's multiple range tests.

Dredge Spoils

James River and Houston Ship Channel dredge spoils were tested by methods perfected in the preliminary test with Kepone-sorbed sediment. James River sediment was collected from mid-channel beneath the Hopewell Bridge; Houston Ship Channel sediment was collected from mid-channel near Carpenters Bayou. Both sediments were collected and handled in accordance with procedures outlined in the EPA-USCE Implementation Manual [4]. Three replicate aquaria received a 5-mm overlay of test spoil, but controls received no additional sediment. Suspended sediment was introduced by the method previously described; however, the suspended material introduced into the control aquaria was prepared from naturally occurring Range Point sediment. Mysid mortality was monitored for 96 hours rather than 10 days, as in preliminary tests. Mortalities in control and exposed aquaria were compared by student's t-tests.

RESULTS

Kepone-Sorbed Sediment

The effect of Kepone-sorbed sediment on mysid survival (Figure 2) was found to be both time ($F = 43$, $df = 9, 27$, $p < 0.05$) and dose ($F = 68$, $df = 3, 27$, $p < 0.05$) dependent. After 10 days, 37% of the mysids exposed to

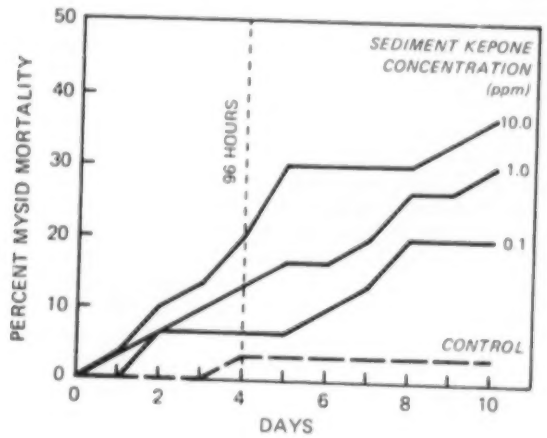


Figure 2. Percent mortality of mysids exposed to control and Kepone-sorbed sediments during a 10-day period.

10.0 ppm Kepone sediment had died: 30% mortality was observed at 1.0 ppm, whereas 20% mortality occurred at 0.1 ppm. Control mortality during this period was only 3%. Significant differences in mortality between controls and the highest concentration ($t = 5.0$, $df = 4$, $\infty = 0.05$) were detected as early as 96 hours.

Oyster shell growth was significantly inhibited ($F = 10$, $df = 3, 12$, $p < 0.05$) at concentrations of 10.0 and 1.0 ppm Kepone–sediment. Oysters exposed to 10.0 and 1.0 ppm averaged 32% less shell growth than did controls, whereas oysters maintained in 0.1 ppm showed only 6% decrease in growth (Figure 3). The actual amount of shell material deposited by oysters averaged 4.03 mm/wk for controls, 3.81 mm at 0.1 ppm, 2.71 mm at 10.0 ppm and 2.73 mm at 1.0 ppm Kepone sediment. No oyster mortality occurred at any exposure concentration.

Substrate reworking by lugworms appeared to be completely inhibited in aquaria containing 10.0 ppm Kepone–sediment. No mortality occurred at any of the concentrations tested. Lugworms exposed to 1.0 ppm and 0.1 ppm reworked surface sediment at rates comparable to controls. Continuous input of suspended material in aquaria made it impossible to quantify substrate turnover rates accurately; however, differences between controls and the highest concentration were obvious (Figure 4).

Oysters and lugworms accumulated Kepone from sediments after 28 days exposure (Figure 5). Oysters exposed to 1.0 ppm Kepone–sediment averaged whole body residues of 0.061 ppm Kepone ($n = 6$); those exposed to 10.0 ppm contained 1.2 ppm. Uptake was nondetectable (<0.02 ppb) in

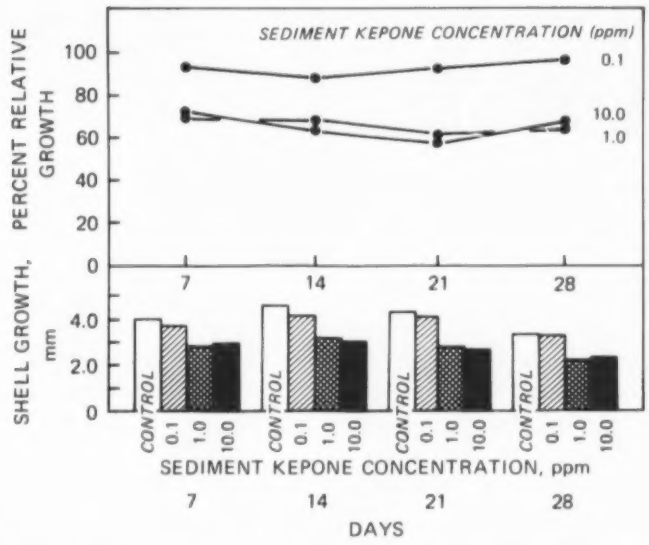


Figure 3. Effect of Kepone-sorbed sediments on oyster shell deposition (percent growth is relative to controls).

oysters exposed to 0.1 ppm Kepone sediment. A dose-dependent relationship was also observed in lugworms. Those exposed to 10.0 ppm contained whole-body residues of 1.1 ppm Kepone. Exposure to 1.0 ppm resulted in 0.46 ppm Kepone in tissues, while lugworms exposed to 0.1 ppm measured 0.043 ppm in tissues.

Macrofauna harvested at 28 days totaled 947 animals, representing 19 species from 4 phyla (Figure 6). Polychaetes were the most abundant organisms collected (672); of these, *Capitella capatata* was the dominant species (433). Polychaetes were almost eliminated from aquaria containing 10.0 ppm Kepone–sediment, while enhancement of polychaetes was observed at 1.0 ppm. This was the only group significantly affected by Kepone-sorbed sediment.

James River Sediment

Mysid survival was not significantly affected by exposure to James River sediment within 96 hours (Figure 7). Although 13% mortality occurred in exposure aquaria, standard bioassay procedures [4,5] allow for up to 10% control mortality. When repeated, mysid survival tests showed less than 10% differences between control and exposed mortalities. Oysters placed on

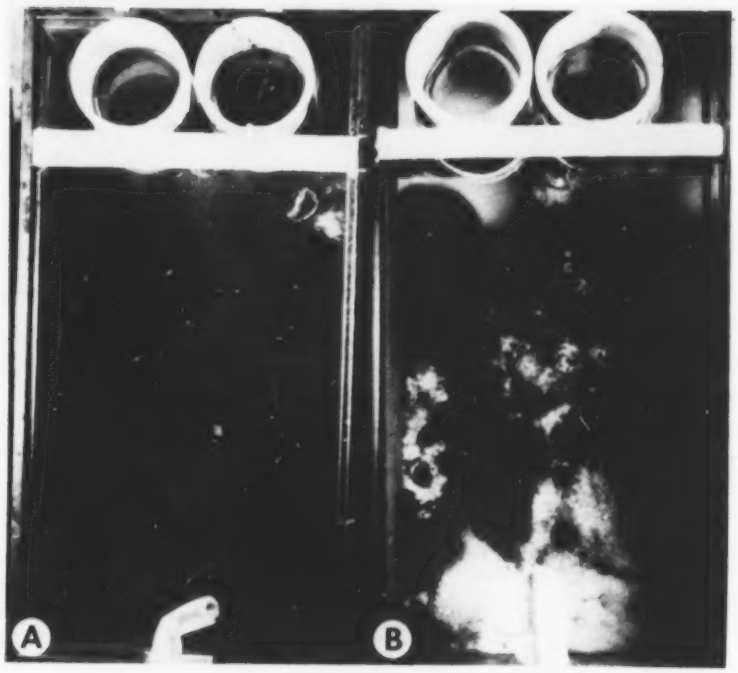
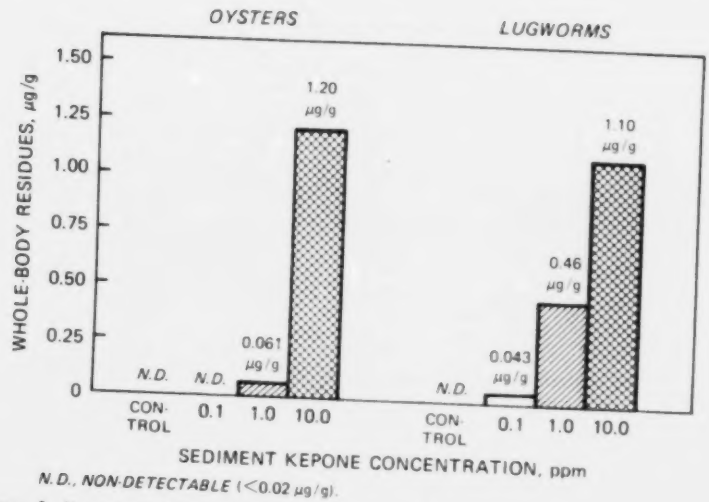


Figure 4. Comparison of substrate reworking by lugworms exposed to 10.0 ppm Kepone-sorbed sediment (A) and controls (B).

James River sediment and exposed to sediment in suspension deposited less shell material than controls throughout 28 days of exposure ($t = 2.70$, $df = 58$, $\infty = 0.05$). The percentage decrease in shell growth of exposed oysters declined steadily throughout the test period (Figure 8). During the fourth week, oysters exposed to James River sediments grew 45% less than controls. Numbers and varieties of settling organisms were not affected; however, this test was conducted during the winter months when local waters were relatively depauperate of planktonic larvae. Lugworm substrate-reworking activity was discernibly reduced in exposed aquaria compared to control aquaria. Lugworms and oysters accumulated Kepone from James River sediments as they did from Kepone-sorbed sediment. Whole-body residues of Kepone averaged 0.51 ppm for oysters ($n = 6$), whereas lugworms averaged 0.75 ppm Kepone in their body tissues at the end of 28 days (Figure 9).



N.D., NON-DETECTABLE (<0.02 µg/g).

Figure 5. Kepone residues in oysters and lugworms following a 28-day exposure to Kepone-sorbed sediments.

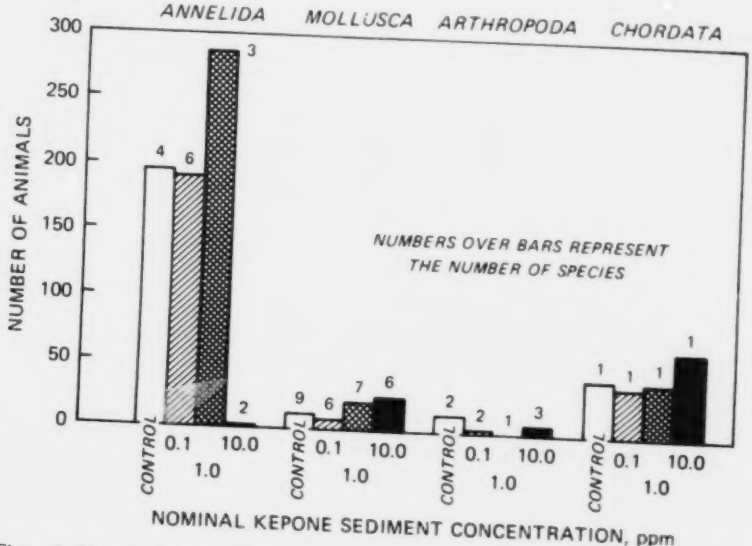


Figure 6. Number of individuals and species collected from exposed and control aquaria after 28 days.

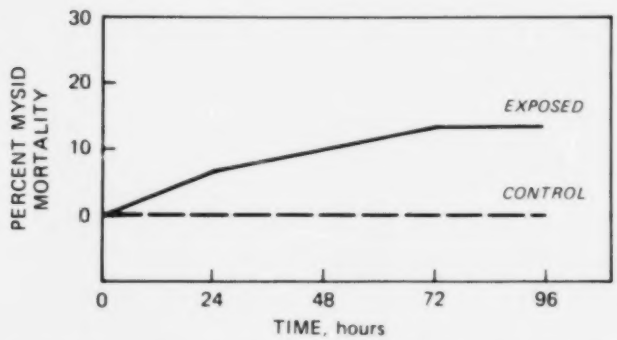


Figure 7. Percent mortality of mysids exposed to James River sediment for 96 hours.

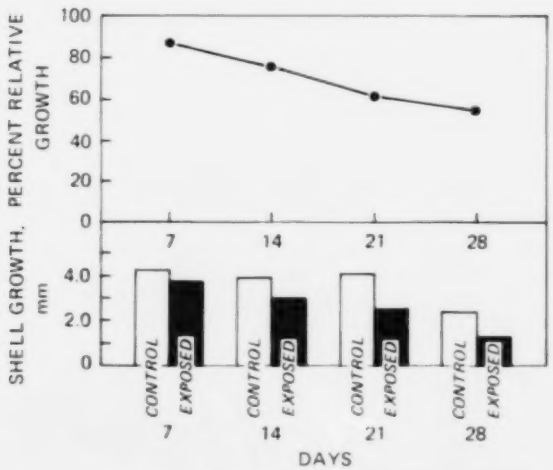


Figure 8. Effect of James River sediment on oyster shell deposition (percent growth is relative to controls).

Houston Ship Channel Sediment

This sediment did not significantly affect mysid survival or oyster shell deposition (Figures 10 and 11). In fact, oyster growth was slightly greater in aquaria containing test sediment. Lugworm activity and the macrofaunal composition in control and exposed aquaria did not differ significantly. Bioaccumulation by oysters and lugworms of specific contaminants in test sediments was not determined.

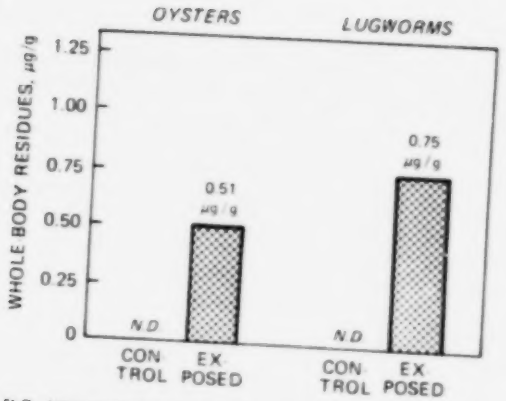


Figure 9. Kepone residues in oysters and lugworms following 28 days of exposure to James River sediments.

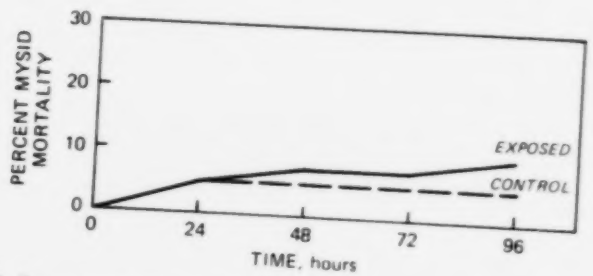


Figure 10. Percent mortality of mysids exposed to control and Houston Ship Channel sediments for 96 hours.

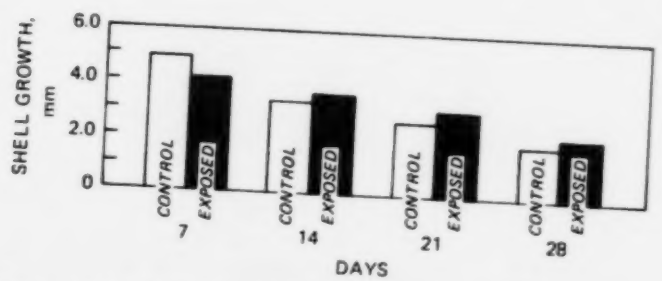


Figure 11. Effect of Houston Ship Channel sediment on oyster shell deposition.

DISCUSSION

In the past, attempts have been made to use bulk chemical analysis [32] of sediments to determine the potential impact of dredged material on the marine environment. However, chemical content does not always reflect actual effects of contaminated sediments because many of the chemical constituents are not in a form that is biologically available to the ecosystem. Therefore, representative marine organisms are used in bioassays as indicators of the environmental activity of xenobiotics associated with dredged material. Bioassays also provide a measure of effect of physical alteration of sediments on indigenous biota at the disposal site.

At present, dredge material bioassays cannot be considered precise predictors of environmental impact due to the number of variables that cannot be addressed in the laboratory, but they do serve as quantative estimators of environmental effects. Consequently, interpretation of bioassay results with respect to permit evaluation is still somewhat subjective. For this reason we used the guidelines currently recommended by the EPA-USCE Technical Committee on Dredge and Fill Material to interpret the results generated by our test. However, several of the proposed test criteria have not been previously addressed by the current implementation manual. For these, i.e., benthic community resiliency and oyster shell deposition, we feel that any significant ($\alpha = 0.05$) differences between control and exposed treatments represent a potentially adverse impact on the marine environment.

Tests conducted with Kepone-sorbed sediment served to illustrate the bioassay protocol. Mysids, oysters and lugworms were significantly affected by test sediments at the highest and, in some cases, the lowest concentration tested. Significant reductions were detected in colonizing populations exposed to the highest concentration. Bioaccumulation of Kepone was found in oysters and lugworms. Based on these findings, introduction of this material into the marine environment even at the lowest concentration could have an adverse impact on marine biota at the disposal site (Range Point).

James River sediment was also found to be unacceptable for release into the marine environment. Although not acutely toxic to test organisms, this material reduced oyster growth and lugworm reworking activity. These sublethal responses reflect physiological effects directly related to the metabolism of the animal. Bioaccumulation of Kepone from James River sediment was detected in lugworms and oysters at concentrations that approximated uptake from 1.0 ppm Kepone-sorbed sediment. (Subsequent analysis of James River sediment measured approximately 0.5–1.0 ppm Kepone.) Uptake of Kepone by marine organisms could ultimately affect human food resources and constitute a potential health hazard. Houston Ship Channel sediment was not harmful to test organisms in terms of the bioassay response criteria.

The bioassay presented in this report provides several distinct advantages over existing dredged material tests. Incorporation of standard bioassay procedures enabled us to compare test results with an extensive data base compiled on a wide variety of marine organisms and toxicants [33]. Use of

culturable test species provides a continuous supply of animals of known ancestry and conditioning and enhances the cost-effectiveness of our procedure by eliminating expensive and time-consuming field collection of bioassay animals. The flow-through seawater design eliminates many artifacts inherent in static bioassay systems, e.g., D.O., metabolite buildup. By combining elutriate, suspended-solid and particulate phases, and addressing the problem of resuspension, we have accounted for synergistic interactions of these phases, producing a much more realistic simulation of actual conditions at the disposal site. Finally, by including benthic community recruitment as a test criterion, we have provided a long-term measure of impact of dredged material on the marine environment.

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